PB81-172298

Epidemiological Study of 'Klebsiella Pneumoniae' Among Pulp and Paper Mill Workers

Wisconsin Univ.-Madison

Prepared for

Health Effects Research Lab.
Cincinnati, OH

Mar 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB81-172298

EPA 600/1-81-023
March 1981

EPIDEMIOLOGICAL STUDY OF KLEBSIELLA PNEUMONIAE AMONG PULP AND PAPER MILL WORKERS

by

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ABSTRACT

This one-year study measured fecal coliform and Klebsiella bacteria densities in several of Wisconsin's pulp and paper mill processing wash waters, treated waters, and waters receiving pulp and paper mill effluent discharge. The isolation of fecal coliform bacteria ranged from as low as the minimum detectable level of <10 organisms/100 ml water sample to as high as an estimated 5×10^6 organisms/100 ml water sample. Water data were obtained from an effluent survey of twenty plants and a within-plant survey of three mills.

The findings from this study support previous investigations concerning the selective growth of coliform bacteria in pulp and paper mills utilizing processed water recycle loops.

It was discovered that several Wisconsin plants operate waste water treatment systems (primary and secondary waste water treatment) that do not effectively reduce bacterial counts and that some allow for bacterial proliferation.

Biochemical testing revealed that over 50% (range from 4 to 97%) of the fecal coliform bacteria associated with pulp and paper mill processing were identified as bacteria of the genus Klebsiella, the most prevalent species of which was found to be K. pneumoniae.

Sampling of paper workers resulted in Klebsiella isolation from the upper respiratory tract. Klebsiella biotyping revealed

that the Klebsiella isolation from a pulp mill worker matched with the same Klebsiella biotype found in the same plant's processing waters.

In addition, a relationship was observed between Klebsiella biotypes isolated from plant sanitary sewers (possible gastro-intestinal carriage) and the same mill's processing waters.

Further study is needed to assess the health hazard risks of populations exposed to water contaminated with Klebsiella from an industrial source.

The currently recognized fecal coliform indicator test selects for a wide range of bacteria, including Kelbsiella from a nonfecal source. This study supports the theory of the fecal coliform test lacking specificity and agrees with the need for reexamining the definition of fecal coliform bacteria to include only an indicator that is consistently and specifically associated with fecal contamination.

ACKNOWLEDGEMENTS

This work was supported by Grant #C3043NAEX from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Environmental Research Center, Cincinnati, Ohio.

I would like to thank the members of my committee, Dr. John Rankin of the Department of Preventive Medicine, Dr. Joseph J. Delfino, head of the Environmental Sciences Section at the Wisconsin State Laboratory of Hygiene and the Department of Civil and Environmental Engineering, and Dr. Marty S. Kanarek of the Department of Preventive Medicine at the University of Wisconsin-Madison for reviewing this work.

I extend special thanks to Dr. Marty Kanarek for serving as thesis chairman and for his guidance, encouragement, and advice during the data collection and writing of this thesis.

Special appreciation is extended to Dr. Joseph J. Delfino and Microbiologist Jon Standridge for the use of the Water Bacteriology Laboratory facilities at the Wisconsin State Laboratory of Hygiene.

I also express my sincere gratitude to Dr. Alfred P. Dufour of the Health Effects Research Laboratory for his tremendous concern and participation with the study by volunteering his services for the Klebocin biotyping as well as for other bacteriological testing.

I wish to thank all of the pulp and paper mills that cooperated with the study. Special thanks is extended to the plant manager and

workers of the groundwood pulp mill for their complete participation and interest in the study. I also thank workers of the Green Bay Park and Recreation Department for their participation in the study.

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I. INTRODUCTION

Industrial organic wastes from pulp and paper mill effluents and their treatment facilities have been known from previous studies to generate high numbers of coliform bacteria. Recently, because of bacterial taxonomic clarifications, the opportunistic pathogen Klebsiella pneumoniae has been isolated and identified as the predominant coliform bacteria from such an industrial waste source. K. pneumoniae is responsible for 2% of the bacterial pneumonia cases in the susceptible population of the entire United States and leads with 60% to 70% of the deaths from this disease. K. pneumoniae is also of increasing importance in hospital-associated infections.

Recycling or reuse of water within the pulp and paper industry has been evaluated with respect to coliform concentrations in various mill systems and has been shown to be selective for bacterial proliferation especially with regard to K. pneumoniae concentrations.

It was the purpose of this study to determine: first, the fecal coliform and Klebsiella bacteria distribution in Wisconsin's pulp and paper mills; and second, because of the potential health risk, to determine if workers employed in pulp and paper mills have significantly greater colonization of Klebsiella than workers employed in nonpulp and paper industries.

II. BACKGROUND

The term coliform bacteria refers to a group of gram negative fermentative rods that inhabit the intestinal tract of humans and other warm-blooded animals without causing disease. Their function has been in indicating fecal contamination of water as well as in indicating the presence of enteric pathogens within the water source. A more restricted group of coliform bacteria exists and is referred to as fecal coliform bacteria; they are exclusive indicators of fecal wastes of warm-blooded animals. A number of investigators have presented evidence demonstrating that high densities of fecal coliform bacteria are present in waters that have not received human or animal wastes (1). It was established that the occurrence of high fecal coliform bacteria identified in several Wisconsin pulp and paper mills are similar to the bacterial problems found all over the nation with industrial effluents containing carbohydrates. Water quality surveys have recently singled out pulp and paper mill wastes as major sources of coliform group organisms (2-6). The coliform bacteria found to be most responsible for the pseudo indication of fecal pollution were members of the bacterial genus, Klebsiella, and the most predominant species identified was K. pneumoniae.

The pulp and paper industry consumes large quantities of fresh water and the amounts utilized range in quantity depending on abundance, cost of fresh water and differences in product requirements of varying mill types. Amounts of water can range from 10,000 gallons

per ton for mills working single products such as groundwood pulp or paper board to 100,000 gallons per ton for integrated mills making several grades of pulp and several grades of paper.

Recently, attention has been focused on reduction of the pollution load of wastes discharged from the pulp and paper mill industries (7). This effort has promoted methods for developing reuse of water within the industry.

Though the reuse of water has been beneficial to the industry via conserving chemicals and fibrous material suspended in the water, utilizing less raw water, conserving heat and minimizing receiving water pollution, there have been indications from previous studies that such reuse of water is selective for coliform bacteria growth (8). Selective growth factors such as bacterial incubating temperatures ranging from 20° to 30°C, sufficient nutrient sources and appropriate pH levels between 6 and 8 can favorably accommodate the coliform growth requirements.

A 1973 EPA study (9) illustrated the recycle processes that occur in typical sulfite pulp mills (see Figs. 1 and 2) and presented data on total bacteria and coliform bacteria counts. The results indicated a positive correlation between high bacterial counts and areas within the plant utilizing recycled water (see Table 1). The reuse of water within plants apparently enables coliform bacteria to enter fresh mill water supplies, thereby inoculating fresh pulp with coliforms which then contaminate following production stages. Though the influent water source to plants may be low in coliform bacteria

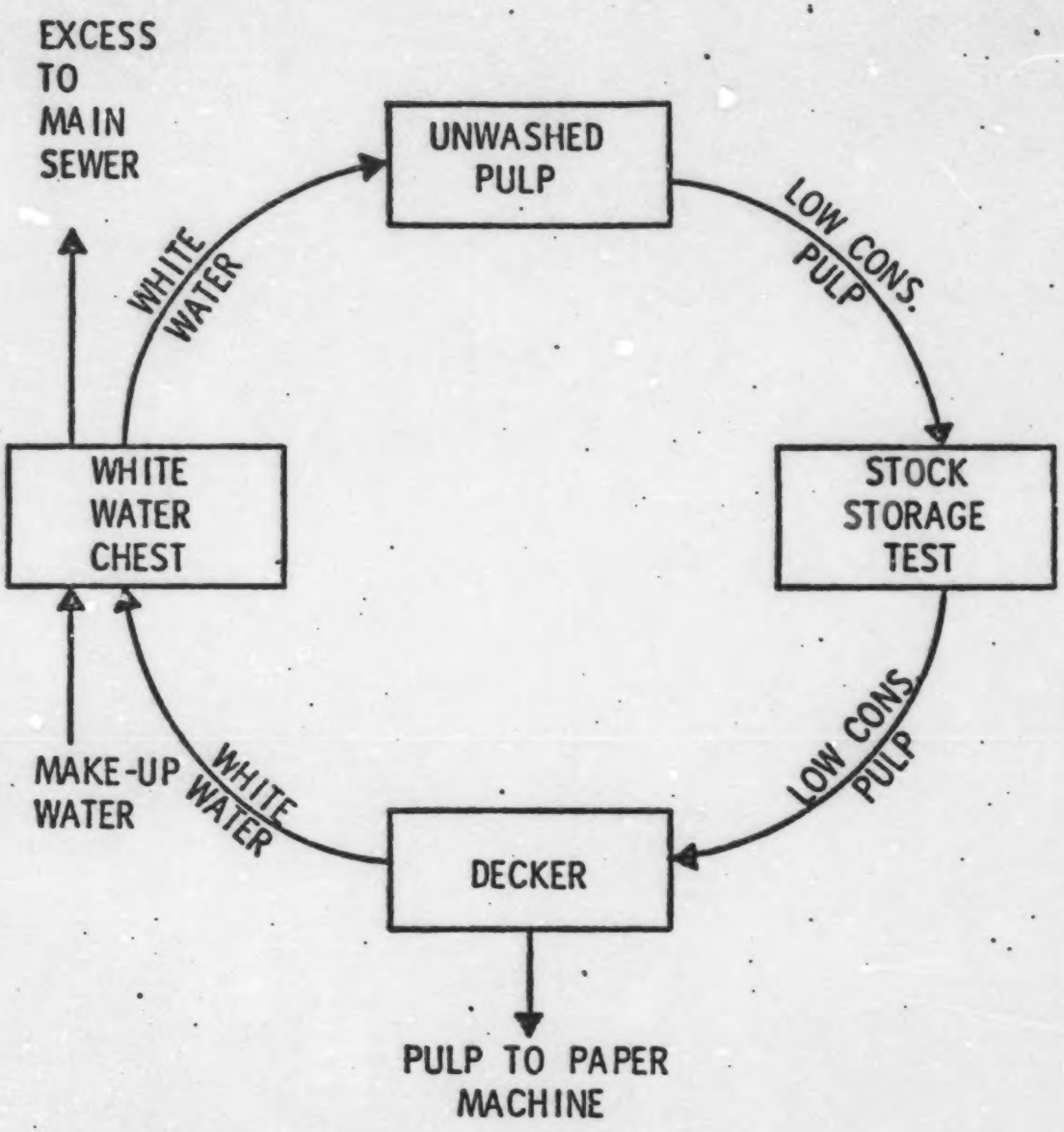

FIGURE 2*

Recycle in Regular Sulfite Pulping System

*S. H. Watkins, EPA-660/2-73-028, p. 16.

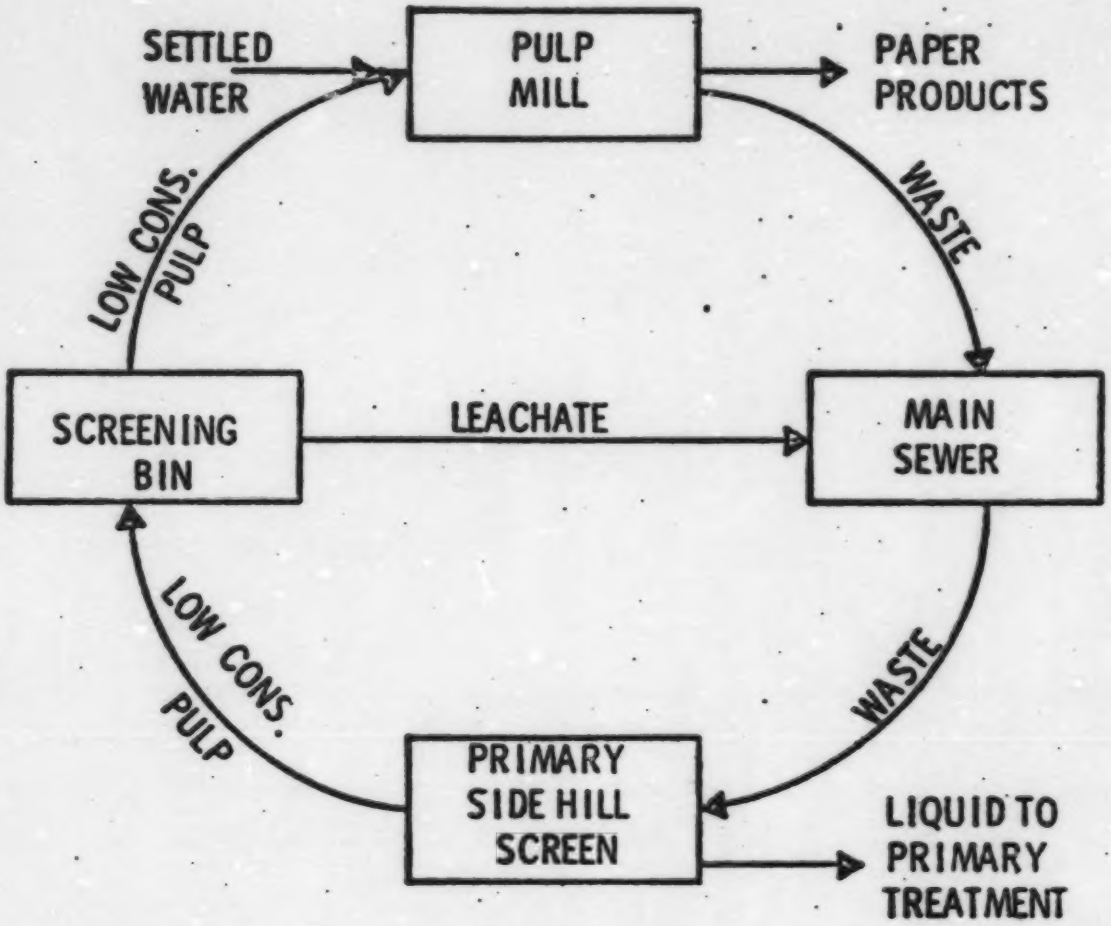

FIGURE 1*.

Recycle in high yield pulping system.

*S. H. Watkins, EPA-660/2-73-028, p. 15.

TABLE 1*

CONCENTRATIONS OF BACTERIA IN MILL WHITE WATER AND TREATMENT SYSTEMS

Source of Sample	Date (1972)	Bacteria/100 ml Total x 10^7	Bacteria/100 ml Colif. x 10^5	Colif. % of Total
Regular sulfite white water storage chest	March 23	2.3	0.19	0.08
	March 23	1.1	80	73
	Oct. 18	2.5	2.8	1.1
#3 reg. sulfite stock chest	Oct. 18	51	0.11	0.002
Reg. sulfite, unscreened stock chest	Oct. 18	0.77	4.2	5.5
Reg. sulfite knotter	Oct. 18	0.08	0.044	0.55
Reg. sulfite decker	Oct. 18	0.32	0.055	0.17
Reg. sulfite Cowan screen	Oct. 18	2.5	2.0	0.80
Sidehill screen in screenings bin	March 23	15	38	2.5
	April 13	6	0.1	<0.017
Screenings bin, north side	March 23	13	340	26
	Oct. 18	280	1700	6.1
Screenings bin, northwest corner	Nov. 6	210	1800	8.6
Screenings bin, west side	March 23	29	740	26
	March 23	5.5	220	40.1
	Oct. 18	11	23	2.1
	Nov. 6	1700	2400	1.4
Screenings bin, leachate	March 23	125	2500	20
	March 23	47	1500	33
	March 24	20	940	47
	March 24	18	260	14
	April 13	10	80	8.0
	April 13	16	60	3.7
	Oct. 18	55	900	16
	Nov. 6	7.7	1.0	0.13
High yield pulp storage chest sawdust	March 24	190	20	0.11
	April 13	10	0.10	0.01
High yield pulp storage chest chips	March 24	660	40	0.06
	April 13	50	1	<0.02
Main sewer after screenings bin	March 24	13	50	3.8
	April 13	7.4	0.20	0.03
Secondary influent	March 24	180	4000	22
	April 13	0.03	1	<33
Secondary effluent	March 24	50	340	6.8
	April 13	31	210	6.8

*S. H. Watkins, EPA-660/2-73-028, p. 17.

levels, influent water can function as a constant source of bacterial inoculum for their subsequent proliferation in recycled water portions of the plant.

Klebsiella pneumoniae is an opportunistic pathogen that is extremely virulent and has quickly developed immunity to almost all antibiotics. As a result of this organism's antibiotic resistance, K. pneumoniae causes 2% of all cases of bacterial pneumonia and it leads with 60% to 70% of the deaths from this disease. In addition to pneumonia, K. pneumoniae is a common cause of urinary tract infections and has been associated with liver abscesses, infant diarrhea, and occulitis. With massive doses of this organism, there may be an increased rate of colonization leading to the increased risk of pathogenic activity.

Studies on the importance of hospital-acquired spread of Klebsiella pneumoniae within hospitals have been increasing during the past 15 years. Its importance as a cause of hospital-associated infection has been amply documented in studies from the Johns Hopkins Hospital (10), Boston City Hospital (11), and elsewhere. These studies have indicated that the most common group of pathogens associated with hospital-acquired infections are of the Klebsiella-Enterobacter group, accounting for approximately 20% of all such infections. Such studies have helped to clarify the extent, distribution and the patterns of hospital-associated Klebsiella infections, but they have not identified the source of the organisms nor the mechanism of hospital-associated spread.

Klebsiella pneumoniae can be routinely isolated from the environment, particularly from nutrient-rich industrial effluents such as pulp and paper mill wastes, textile finishing plant wastes, and sugar cane wastes. Duncan and Razzell (12) evaluated samples of water, soil and bark from three different forest environments and effluent samples from a pulp and paper mill for total and fecal coliform bacteria. They found that only a small percentage of the bacteria isolated was *E. coli* and that up to 71% of the isolates were identified as *Klebsiella*. Bauer (13) examined pulp and paper mill effluents for total and fecal coliform bacteria. He found coliform densities as high as 1.6×10^8 coliforms per 100 ml and fecal coliform densities as high as 4.2×10^6 organisms per 100 ml. Of 300 isolated fecal coliforms, he found 68% to correppond biochemically to organisms of the *Klebsiella-Enterobacter* group. Further evaluation of the isolates showed 98% to be identified as *K. pneumoniae*. Knittel et al. (14) examined total coliform bacteria in pulp and paper mill effluents and found levels in excess of 10^5 organisms per 100 ml. Further evaluation showed 60% to 80% of these isolates to be identified as *Klebsiella*. Extensive field studies carried out in three widespread geographical areas of the U.S. by the National Council Staff of the National Council of the Paper Industry for Air and Stream Improvement (15) have further verified the widespread distribution of *Klebsiella* in surface waters receiving effluent from pulp and paper mills. Mallman (16) examined the coliform biotypes in Lake Erie waters receiving discharge from a city's untreated primary effluent and

three paper mills. He found that nearly 50% of fecal coliform isolated belonged to the Klebsiella-Enterobacter group. Huntley et al. (17) further confirmed the presence of large numbers of Klebsiella organisms in waters receiving pulp mill effluents. A total of 60% of the isolates were identified as Klebsiella.

Klebsiella has also been isolated from drinking water emanating from redwood storage tanks (18). An Oregon survey was made of the bacteriological quality and chlorine content of 33 public and private water systems that utilized redwood storage tanks. Coliforms of the genus Klebsiella and Enterobacter were isolated from 9 out of 10 private drinking water systems and from 11 out of 23 water systems in state and federal parks. The total coliform counts in the private systems exceeded federal membrane filter guidelines--zero organisms per 100 ml water sample--by as much as 10 to 40-fold. Coliform counts were highest in the newer reservoirs, i.e., tank systems operating for less than one year. Wooden reservoirs exert a high chlorine demand and 0.4 ppm chlorine residual in the incoming tank water proved inadequate. A surprising observation was the repeated occurrence of coliforms after refilling the tanks with chlorinated municipal water and even after scrubbing with a solution of 200 ppm of chlorine. This recurrence could only come about if the coliforms were uniquely chlorine resistant or if the chlorine demand of the wood was sufficiently high to rapidly reduce its effectiveness. Further testing showed a continual occurrence of chlorine-sensitive coliforms after the addition of chlorinated water (up to 200 ppm).

Such information appears to indicate that a continual source of Klebsiella is entering the tank water from wood microenvironments not accessible to dissolved chlorine. It was suggested by Seidler et al. (18) that specific water soluble nutrients in redwood (as in other numerous types of botanical material) induce a natural selection for the coliforms of the genus Klebsiella.

Dufour and Cabelli (19) examined textile finishing plant wastes for total coliform and Klebsiella bacterial counts. Water samples taken upstream from the plant contained coliforms with levels of 300 coliforms per 100 ml and a Klebsiella range of 130 organisms per 100 ml. The coliform density downstream from the plant was four orders of magnitude greater than that above it, with levels of 3.7×10^6 coliforms per 100 ml and Klebsiella made up approximately 50% of the coliform population with a range of 1.7×10^6 organisms per 100 ml. The number of coliforms in the plant effluent was six orders of magnitude greater than that found in the upstream waters with levels of $2.3 \times$ coliforms per 100 ml and more than half of these were Klebsiella with a range of 1.3×10^8 organisms per 100 ml.

Bagley and Seidler (20) found that the environmentally derived isolates of Klebsiella are phenotypically and serologically indistinguishable from Klebsiella isolates obtained from human and animal infections. This would suggest that effluent strains of isolated Klebsiella are as hazardous to human health as those Klebsiella strains actually isolated from clinical cases. Dufour and Cabelli (19) isolated relatively large numbers of Klebsiella pneumoniae from

industrial effluent water with capsular types 1 to 6. Historically, K. pneumoniae capsular types 1 to 6 have been associated with respiratory tract infections. Isolates of Klebsiella from industrial effluents are not distinguishable from isolates of Klebsiella from clinical sources, and if colonization can occur from exposure to water containing high densities of Klebsiella, then there may be a potential health hazard associated with the occupational exposure to such water. Epidemiological investigations are necessary to determine the potential health significance of Klebsiella obtained in the working environment.

There have not been any published studies to date relating the incidence of illness among paper mill workers whose work environment has long been known to contain levels of indicator bacteria which exceed existing water quality standards as compared to workers employed in industries without this risk of bacterial exposure.

III. MATERIALS AND METHODS

Study Areas for Water Sampling

Wisconsin pulp and paper mills located predominantly in the north central and northeastern part of the state were chosen as sites for the bacteriological examination of industrial waters within their processing stages and waters receiving their effluent discharges. Figure 3 shows the location and type of each sampled mill in the study.

Beginning July 1, 1979, this study proceeded with a telephone survey to all Wisconsin pulp and paper mill plant officials, to discuss the applicability of each plant's participation in this study. However, after meetings and discussions with all of Wisconsin's 36 pulp and paper mills, only three of the 20 sampled mills gave permission to extend water sample collection to within the plant's processing stages. Meetings were also attended with representatives of the Wisconsin Paper Council and members of the National Council of the Paper Industry for Air and Stream Improvement; however, there was no increase in the number of plants cooperating with the study. The three mills that were examined from within the plants included: a groundwood pulp mill, a deink pulp and fine papers plant, and a sulfite pulp and fine papers plant. Of the 20 sampled mills, 17 were collected by the Wisconsin Department of Natural Resources and included influent to treatment facilities and also effluent samples. The processing types among the 20 mills included: tissue paper,

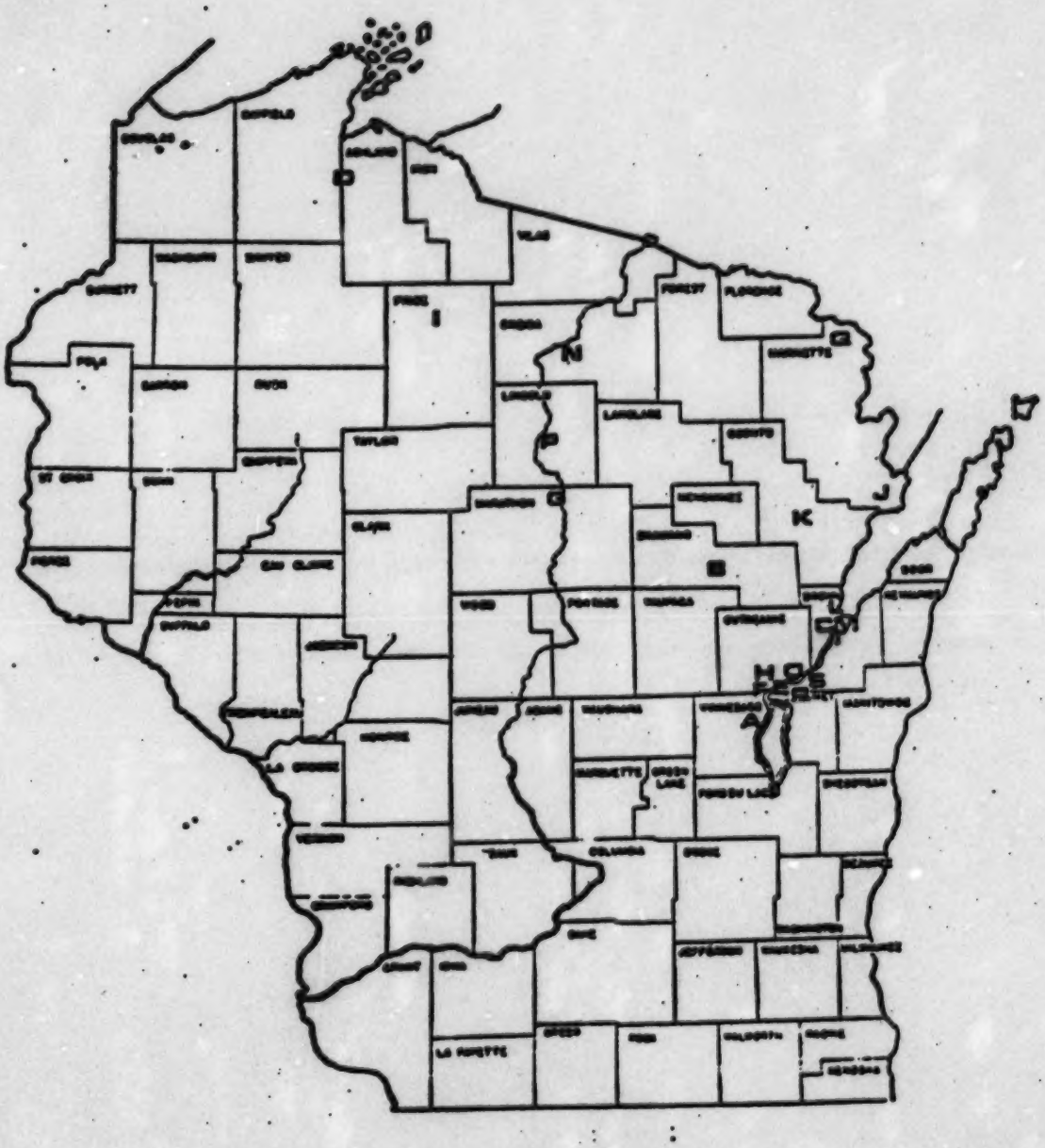

FIGURE 3

LOCATIONS OF SAMPLED PULP AND PAPER MILLS IN WISCONSIN

FIGURE 3 (continued)

Key for plant types in the study:

A = Tissue paper

B = Tissue paper

C = Tissue paper (glassine)

D = Deink pulp and tissue

E = Deink pulp and tissue

F = Deink pulp and fine papers

G = Deink pulp and fine papers

H = Sulfite pulp

I = Sulfite pulp and fine papers

J = Sulfite pulp and fine papers

K = Sulfite pulp and tissue

L = S-lfite pulp and tissue

M = Sulfite, deink pulp tissue paper

N = Sulfite pulp and glassine papers

O = Kraft pulp and specialty papers

P = Groundwood pulp

Q = Groundwood pulp and book paper

R = Groundwood pulp and book paper

S = Chemical-mechanical pulp, denik pulp and fine papers

T = N.S.S.C.* and packaging medium

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*Neutral Sulfite Semichemical pulp.

tissue papers (glassine), deink pulp and tissue, deink pulp and fine papers, sulfite pulp, sulfite pulp and fine papers, sulfite pulp and tissue, sulfite and deink pulp tissue paper, sulfite pulp and glassine, kraft pulp and specialty, groundwood pulp, groundwood pulp and book paper, chemical-mechanical deink pulp and fine papers, and N.S.S.C.* and packaging medium plants.

Sample Collection

Three mills allowed water sampling from within each plant. Collected samples were processed according to Standard Methods for the Examination of Water and Wastewater (SM)(21). The Wisconsin Department of Natural Resources submitted influent to treatment and effluent water samples from 17 mills. The samples were also collected and processed according to SM (21).

Water samples containing residual chlorine were collected in sterile bottles containing a dechlorinating agent--namely, sodium triosulfate,** which neutralized residual chlorine and prevented continued bactericidal action of the chlorine during the time the sample was in transit to the laboratory.

Samples were collected leaving ample air space in the bottle (at least 2.5 cm) to facilitate mixing of the sample by shaking preparatory to examination. The sample bottles were maintained at

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*Neutral Sulfite Semichemical pulp.

**Approx. concentration of 100 mg/l per sample bottle, enough to neutralize approx. 15 mg/l of residual chlorine.

approximately 4°C during shipment to the laboratory where bacteriological examination was initiated within 24 hr of sample collection.

Fecal Coliform Response

Determination of fecal coliform bacterial densities was made by a membrane filter technique. The membrane filter procedure calls for an enriched lactose medium that depends on an incubation temperature of 44.5 ± 0.2°C for selectivity. The M-FC* medium utilized for this test was prepared according to the media specifications in section 905C, No. 10, of SM (21).

The volume of water sample examined by the membrane filter technique involved filtering (using 0.45 μm-pore-size membrane filters) serial dilutions of sample to yield a countable membrane, usually counts between 20 and 60 fecal coliform colonies per filter.

Prepared culture plates were then sealed in waterproof plastic bags and submersed in a 44.5 ± 0.2°C waterbath for 24 hours. The plates were anchored below the water surface during incubation to maintain critical temperature requirements. Colony numbers and color were recorded on each plate after incubation.

All fecal coliform isolates (blue colonies on M-FC medium) were verified by subculturing isolated blue colonies to EC broth at 44.5°C for 24 ± 2 hr. Gas production in the fermentation tube within 24 hr or less was considered a positive reaction indicating fecal origin.

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*Membrane-fecal coliform.

Klebsiella Response

A membrane filter method for the enumeration of Klebsiella in fresh and marine waters was developed by Dufour and Lupo (22). Appropriate volumes of water samples were filtered using 0.45 µm-pore-size membrane filters and incubated at 35°C for 48 hr on the specifically developed MK medium. All nonpink colonies were counted for total Klebsiella evaluation.

The relative recovery of the method against a noninhibitory reference medium for five Klebsiella strains was found to average about 75%. The medium was also found to be highly selective where Klebsiella was detectable even when comprising less than 0.001% of the coliform population. Presumptive speciation of the isolated Klebsiella was made by using triple sugar iron agar, urea slants, Simmons' citrate agar, lysine and ornithine decarbosylase broths, motility and the indole, methyl red and vogues-proskauer tests (IMViC).

Further identification and characterization of the Klebsiella included the API 20 E system and the bacteriocin (Klebocin) sensitivity typing of Klebsiella according to Buffenmeyer et al. (23), tested by A. Dufour, Marine Field Station, Health Effects Research Laboratory, CIN, West Kingston, Rhode Island.

Fecal coliform-positive Klebsiella were identified by subculturing isolated Klebsiella colonies from MK agar into EC tubes as previously described.

In cases where total Klebsiella counts were not obtainable, underestimated counts of fecal coliform-positive Klebsiella were enumerated by subculturing typically large, raised, mucoid blue fecal coliform colonies onto EMB (eosin methylene blue) plates for incubation at 35°C for 24 hrs. Typically large, raised, mucoid pink colonies with purple centers were isolated and underwent a series of biochemical reactions (see above) for presumptive speciation of Klebsiella. Presumptive Klebsiella colonies were then picked from the EMB plates into EC tubes for fecal coliform response. Gas production was considered to be positive for fecal coliform response.

Biochemical Testing of the Klebsiella Isolates

The IMViC series of tests used in the differentiation of the coliform bacteria consists of indole, methyl red, vogues-proskauer and citrate tests. Figure 4 illustrates the protocol for Klebsiella isolation.

Indole Test

Tryptophane broth was used as described under Media Specifications, No. 21, in SM (21) and then dissolved 5g paradimethylbezaldehyde in 75 ml isoamyl (or normal amyl) alcohol. To this, 26 ml conc. HCl was added. The amyl alcohol solution should have a pH value of less than 6.0. Five ml portions of the medium were inoculated from a pure culture and incubated at 35 ± 0.4°C for 24 ± 2 hr. Add 0.2 to 0.3 ml test reagent and shake. Let the tube stand for about 10 min and observe the results. A dark red color in the amyl alcohol

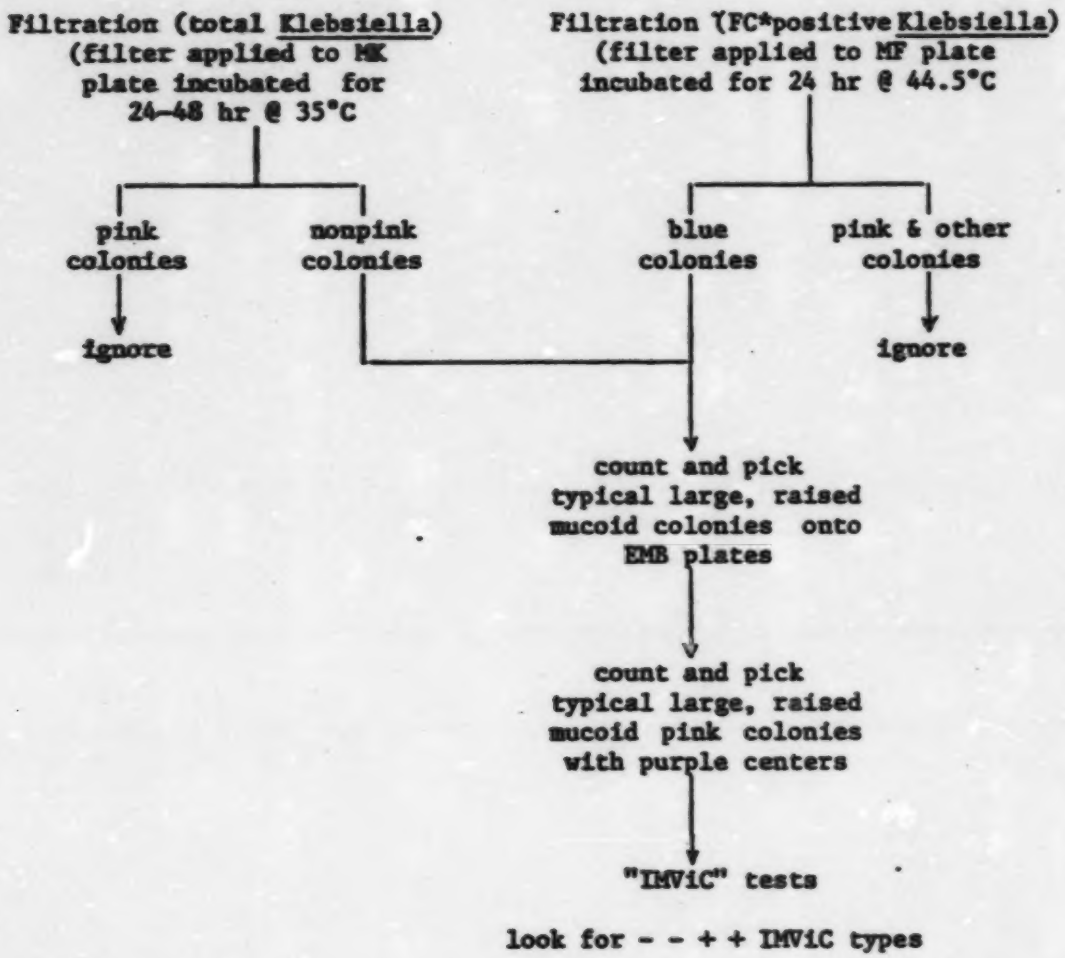

FIGURE 4

PROTOCOL FOR THE ISOLATION OF KLEBSIELLA

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*Fecal coliform.

surface layer constitutes a positive indole test; the original color of the reagent, a negative test. An orange color probably indicates the presence of skatole and was reported as a ± reaction.

Methyl Red Test

Buffered glucose broth was used as described under Media Specifications, No. 22, in SM (21), and then dissolved 0.1 g methyl red in 300 ml of 95% ethyl alcohol and diluted 10 ml portions of medium from a pure culture. Incubate at 35°C for 5 days. To 5 ml of the culture add 5 drops methyl red indicator solution. Record a distinct red color as methyl red positive and a distinct yellow color as methyl red negative. A mixed shade was recorded as questionable and possibly indicative of incomplete culture purification.

Vogues-Proskauer Test

This test may be carried out using the medium as described for the methyl red differential test or, if desired, an alternative salt peptone glucose medium as described under Media Specifications, No. 23, in SM (21). Then 5g purified alpha-naphthol (melting point 92.5°C or higher) was dissolved in 100 ml absolute ethyl alcohol. Forty grams of KOH was then dissolved in 100 ml distilled water. Five ml of culture medium was then inoculated and incubated at 35 ± 0.5°C for 48 hr. To 5 ml of the culture add 0.6 ml naphthol solution and 0.2 ml KOH solution. Development of a pink to crimson color in the mixture from 2 to 4 hr after adding the reagents was considered a positive test.

Sodium Citrate Test

Simmons' citrate agar was used as described in SM (21) under heading No. 25, then the agar medium was inoculated with a straight needle, using both a stab and a streak. Incubate at 35 ± 0.5°C for 48 hr. Growth on the medium with (usually) a blue color constituted a positive reaction; the absence of growth was recorded as negative.

Additional tests are required for the separation of Klebsiella and Enterobacter. Speciation of Klebsiella is still not clear; however, Edwards and Ewing (24), Cowan, Bascomb et al. and Johnson et al. (25) accept that K. pneumoniae and E. aerogenes can be distinguished by motility and decarboxylation of ornithine.

Urea Test

Christensen urea agar base was used where 1 g peptone, 5 g sodium chloride, 1 g dextose, 2 g monopotassium phosphate, 0.012 g phenol red, and 20 g urea are added to 100 ml distilled H_2O. Adjust pH to 6.8. Add 15 g agar, autoclave at 121"C for 15 min. Inoculate entire surface of slant and incubate at 35°C. Examine after 24 hr incubation. Urease-positive cultures produced an alkaline reaction as observed by a red color.

Decarboxylase Test

Moeller broths were used where 5 g peptic digest of animal tissue, 5 g beef extract, 0.0.0 g bromocresol purple, 0.005 g cresol red, .5 g dextrose and 0.005 g pyridoxal are added to 1 liter of distilled H_2O. Adjust pH to 6.0. Divide broths into three parts:

one as a control, then add 1% L-lysine dihydrochloride and L-ornithine dihydrochloride to the remaining portions. Alternatively, add 2% DL-Amino acids. Readjust pH of ornithine portion. Autoclave tubes at 121°C for 10 min. Inoculate test and control tubes with culture from plate. Cover with a 4 to 5 mm layer of sterile mineral (paraffin) oil. Incubate at 37°C for 24-48 hr. Positive reactions were recorded as alkaline or having purple color.

Motility Test

Combine 3 g beef extract, 10 g peptone, 5 g sodium chloride, and 4 g agar to 1 liter distilled H_2O. Adjust pH to 7.4. Autoclave at 121°C for 15 min. Inoculate agar with straight stab of culture on wire. Incubate at 35°C for 24-48 hr. Growth away from original stab into surrounding agar is recorded as positive for motility.

For K. pneumoniae, look for urease +, lysine decarboxylase +, ornithine decarboxylase -, and motility -.

Bacterial Cultures Obtained from Workers Assayed for Klebsiella Response

A cohort of 25 workers from a groundwood pulp mill volunteered to allow bacterial specimen acquisition to determine their Klebsiella colonization status.

Nasal specimens were obtained with a cotton calcium alginate swab on a flexible wire which was passed gently into each of the nares, rotated and removed. Throat specimens were obtained with a cotton swab from each tonsillar area. Both swabs were inoculated

into separate modified Stuart's transport medium (MST), the same type as is used by the Wisconsin State Laboratory of Hygiene for mailed clinical specimens. The tubes were packed in styrofoam containers containing ice bottles until delivery to the laboratory for bacterial enumeration within 24 hr of sample collection.

In order to avoid overdilution of the obtained specimens, quantification of the organisms was limited to direct swab streaking for bacterial isolation on EMB plates incubated at 35°C for 24-48 hr. Swabs were mixed in the transport media, "wrung-out" on the inside of the tube and streaked where approximately 0.1 ml of culture was delivered to the plate. Plates without bacterial isolations were considered negative only after a maximum incubation period of 72 hr. Plates containing typically large, raised, mucoid pink colonies with purple centers were isolated and presumptively speciated using the previously listed biochemical tests. Further characterization and biotyping of the isolated Klebsiella was evaluated by A. Dufour at the Health Effects Research Laboratory-CIN, West Kingston, Rhode Island.

Bacterial Examination of Plant Sanitary Waste Waters

It was possible to obtain samples of sanitary waste waters from two pulp and paper mill companies. Samples were collected in sterile sampling bottles, shipped under 4°C and processed at the Wisconsin State Laboratory, Water Bacteriology Unit, within 24 hr of sample collection. All samples were serially diluted and processed

via the membrane filtration technique using MF agar plates for fecal coliform evaluation and MK agar plates for totai Klebsiella enumeration. Klebsiella isolates were verified for fecal coliform response by subculturing isolates to EC broth for 24 hr at 44.5°C. Further characterization of isolated Klebsiella was obtained utilizing the biochemical reaction patterns (as previously described) and, through biotyping, for the presumptively identified Klebsiella isolates.

IV. RESULTS

Table 2 summarizes the fecal coliform and Klebsiella responses of all of the sampled pulp and paper mill influent to treatment and plant effluent waters. The levels of fecal coliform bacteria isolated and identified as Klebsiella ranged from as low as <10 organisms/100 ml (minimum detectable level) to as high as 3×10^6 organisms/100 ml. Previous studies that examined pulp and paper wastes have found Klebsiella bacteria to comprise from 50° to >90° of the total coliform population and from 18% to 45° of the fecal coliform population (25).

The presence of high Klebsiella counts in the fecal coliform analyses of the study in the absence of E. coli suggests that bacterial "regrowth," initially considered to occur only among non-fecal coliforms, now can be applied to certain biotypes of Klebsiella that multiply in waters contaminated with wastes from wood pulping processes and meet the criterion for fecal coliforms. Table 2 also indicates that industrial waste water treatment has limited effects on reducing bacterial counts, especially on Klebsiella.

Industrial waste waters have federal regulations limiting suspensed solid levels and biochemical oxygen demand loads although no limitations exist for bacterial pollution. Because of the finding that high levels of FC* positive Klebsiella (i.e., EC gas-positive) are present in pulp and paper mill treated waters and subsequent

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*Fecal coliform.

TABLE 2

FECAL COLIFORM (FC) AND KLEBSIELLA RESPONSES OF INFLUENT TO TREATMENT AND EFFLUENT WATER SOURCES OF WISCONSIN PULP AND PAPER MILLS

Plant Types	Water Source	FC Response /100 ml	Total Klebsiella /100 ml	FC identified as Klebsiella	% FC* identified as Klebsiella
Tissue paper plant A	Influent to treatment (1)	30,000 EST	10,000	5,000	17
	Effluent	<10	<10	<10	
Tissue paper plant B 1st collection	Effluent (grab)	<10	<10	<10	
2nd collection	Effluent (grab)	150	400	40	27
Tissue papers (glassine)	Influent to treatment (2)	7,500	--	3,600	48
	Effluent	4,800	--	2,900	60
Deink pulp & tissue Plant A	24 hour flow/Prop. composite effluent	1,300	--	50	4
Deink pulp & tissue Plant B	Influent to treatment (3)	7,000	15,000	3,500	50
	Effluent	3,100	12,000	1,300	42
Deink pulp & fine papers Plant A	Effluent	50		40	80

TABLE 2 (continued)

Plant Types	Water Source	FC Response /100 ml	Total Klebsiella /100 ml	FC identified as Klebsiella	% FC* identified as Klebsiella
Deink pulp & fine papers Plant B	Influent to Plant B	490		<10	
	Influent to treatment (4)	$3x10^6$EST		$2x10^6$EST	67
	Effluent	$2x10^6$EST		$1x10^6$EST	50
Sulfite pulp 1st collection	Influent to treatment (5)	3,800		1,000	26
	Effluent	$5x10^6$EST		$3x10^6$EST	60
Sulfite pulp 2nd collection (after 24 hr shutdown)	Influent to treatment (5)	$2x10^6$EST	$1x10^6$EST	$5x10^5$	25
	Effluent	$2x10^6$EST	$1x10^6$EST	$8x10^5$	40
Sulfite pulp & fine papers	Effluent Plant A	<10		<10	
Sulfite pulp & fine papers Plant B	Influent to Plant B	<10		<10	
	Influent to treatment (6)	1,700		1,000	59
	Effluent	50,000		45,000	90
Sulfite pulp & tissue Plant A	Effluent	6,200		5,000	81

TABLE 2 (continued)

Plant Types	Water Source	Response /100 ml	Total Klebsiella /100 ml	FC identified as Klebsiella	% FC* identified Klebsiella
Sulfite pulp & tissue	Effluent	30,000	20,000	10,000	33
Plant B					
Sulfite, & deink pulp tissue	Effluent	12,000		6,000	50
Sulfite pulp & glassine	Effluent (grab)	950		430	45
Kraft pulp & specialty papers	Primary influent to treatment (7)	310		200	65
	Primary effluent (from primary clarifier)	150		100	67
	Effluent	<10		<10	
Groundwood (internal close-up)	Influent to plant	10		10	
	Effluent (clarifier white H_2O)	60		40	67
Groundwood pulp & book paper	Influent to treatment (8)	185,000		150,000 EST	81
Plant A					
Groundwood pulp & book paper	Influent to treatment (9)	5,500		4,600	84
Plant B	Effluent	1,500	3,000		

TABLE 2 (continued)

Plant Types	Water Source	Response /100 ml	Total Klebsiella /100 ml	FC identified as Klebsiella	% FC* identified as Klebsiella
Chemical-pulp, deink pulp & fine papers	Influent to treatment (10)	11,000	15,000	9,000	82
	Effluent	330	5,000	250	76
N.S.S.C. & packaging medium**	Influent to treatment (11)	110		70	64
	Effluent	240		180	75

*As measured from FC bacteria counts.

**Neutral sulfite semichemical pulp.

(1) = primary clarifier.

(2) = primary clarifier plus settling lagoon.

(3) = primary clarifier and 2-stage activated sludge with centrifuges for sludges.

(4) = primary clarifier and 2-stage activated sludge with holding lagoon for sludges.

(5) = primary clarifier and secondary activated sludge system.

(6) = primary clarifier, trickling filter, secondary clarifier and mechanized sludge dewatering.

(7) = primary clarifier, pure O_2 activated sludge and polishing lagoons.

(8) = deep channel aeration.

(9) = primary clarifiers and 2-stage activated sludge.

(10) = primary clarifier and pure O_2 activated sludge system.

(11) = internal close-up and recycle utilizing reverse osmosis and evaporation/recovery.

effluent waters, the validity of the fecal coliform test as a specific indicator of fecal waste pollution must be reevaluated.

A 2 x 2 contingency table was used to test the null hypothesis that Klebsiella isolation from 9 plants was independent of whether the water source proceeded to treatment or was discharged from final treatment.

	Water prior to treatment	Water discharged after treatment	Total
Klebsiella isolation	9	6	15
No Klebsiella isolation (i.e.,<10 organisms /100 ml)	0	3	3
Total	9	9	18

$\chi^2 = 3.6 < \chi^2 (\alpha = .05) = 3.84$. Thus, the hypothesis that isolating Klebsiella is independent of water source was accepted--i.e., treatment of waste water has no significant effect in reducing Klebsiella levels. This test shows that the effluent treatment facilities at each plant had no effect in reducing Klebsiella bacteria levels being dischraged as final plant effluent.

Table 3 summarizes the FC and Klebsiella responses of a groundwood pulp mill utilizing the membrane filter method for isolation. The levels of fecal coliforms identified as Klebsiella ranged from as low as <10 organisms/100 ml to as high as 3×10^6 EST or 75%.

TABLE 3

FECAL COLIFORM AND KLEBSIELLA RESPONSES OF A WISCONSIN GROUNDWOOD PULP MILL

Water Source	Fecal Coliform/100 ml	FC identified as Klebsiella/100 ml	% FC identified as Klebsiella
Influent water to plant	<10*	<10	
Raw wood** from debarker extracted with sterile phosphate buffer	850	600	71
Grinder white water	3,500	1,600	46
White water chest	400,000	240,000	60
Growth on sides of white water chest	$3x10^6$EST	$2x10^6$EST	67
Thin stock	38,000	23,000	61
Hydrapulper	$4x10^6$EST	$3x10^6$EST	75
Ground pulp near wet machine	24,000		
Wet machine	330,000	230,000	70
Clarified white water	60	40	67

*<10 = minimum detectable levels.

**Aspen wood.

Figure 5 illustrates a typical flow diagram for a groundwood pulp mill. As is apparent from the high fecal coliform and Klebsiella counts within the plant's white water chest, growth on the sides of the white water chest, and hydrapulper (with correspondingly low bacterial counts entering the plant via fresh water inoculum), the association between selective bacterial growth and recycled water systems becomes clear. From the samples taken, it can be interpreted, although not with complete certainty, that the source of the bacteria was from the wood chips and soil that initially came into the mill system. The logs utilized are primarily an aspen-wood type located in close proximity to the mill. Soil can readily contaminate the wood because of the "dragging out" process of moving wood from cutting areas to yarding areas for loading preparations. The highest bacterial count found in the mill corresponded to the excess water source which overflowed into a hydrapulper awaiting to be reused. The retention period in this tank was apparently sufficient to allow bacteria to incubate under the appropriate temperature and nutrient requirements needed for growth. Temperatures within the tank were found to range between 15° to 25°C. Wood fibers and particulate matter were found in the holding tank so that a nutrient source was available from fiber leachate which could have provided the needed enrichment for bacterial growth.

The plant sanitary sewers were separate from the plant processing waste water sewers so that fecal contamination of the plant processing waters by plant sanitary wastes was highly unlikely.

However, of interest was that fecal coliform bacteria were isolated in high densities from within the plant processing waters. Also of interest was that Klebsiella comprised up to 75% of the fecal coliform bacteria. The high levels of thermotolerant Klebsiella from an apparent nonfecal source is further evidence of the need to reevaluate the fecal coliform indicator test for applications to such industrial waste waters.

Fecal coliform bacteria of which Klebsiella predominated were consistently isolated from other mill process types indicating the ubiquity of Klebsiella in the pulp and paper mill industry. Figure 6 is a schematic flow diagram of the sampled deink pulp and fine paper mill. Table 4 sujmarizes the FC and subsequent Klebsiella responses of the sampled deink pulp and fine papers plant.

It was apparent that water flowing into the plant had relatively low levels of fecal coliform bacteria and no isolation of Klebsiella was made. However, the effluent chest water consisting of process waste water from the side hill washers and tile tank may have been the apparent source for fecal coliform bacteria of which Klebsiella accounted for >90%. Complications concerning control of bacterial pollution becomes apparent when the levels of bacteria are examined from the stock going into the decker chest (which originates from the bleach tower) and from the decker chest itself. Bleach treatment of stock alone (typically single-stage hypochlorite bleaching at terminal pH range between 6.0 and 7.0 with temperature ranges between 25° to 35°C) is apparently not sufficient for bacterial

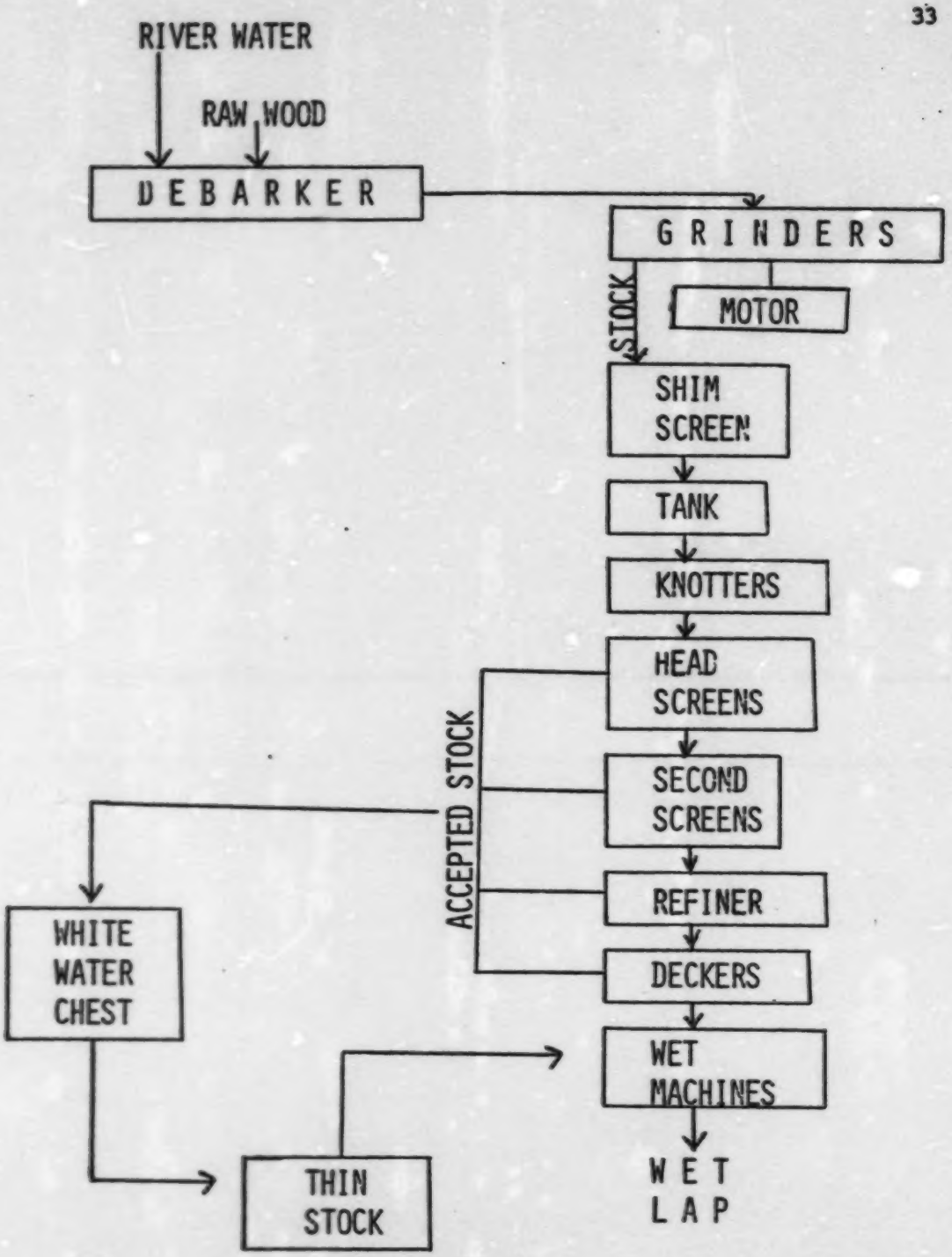

FIGURE 5

SCHEMATIC FLOW DIAGRAM OF BASIC GROUNDWOOD PULP MILL

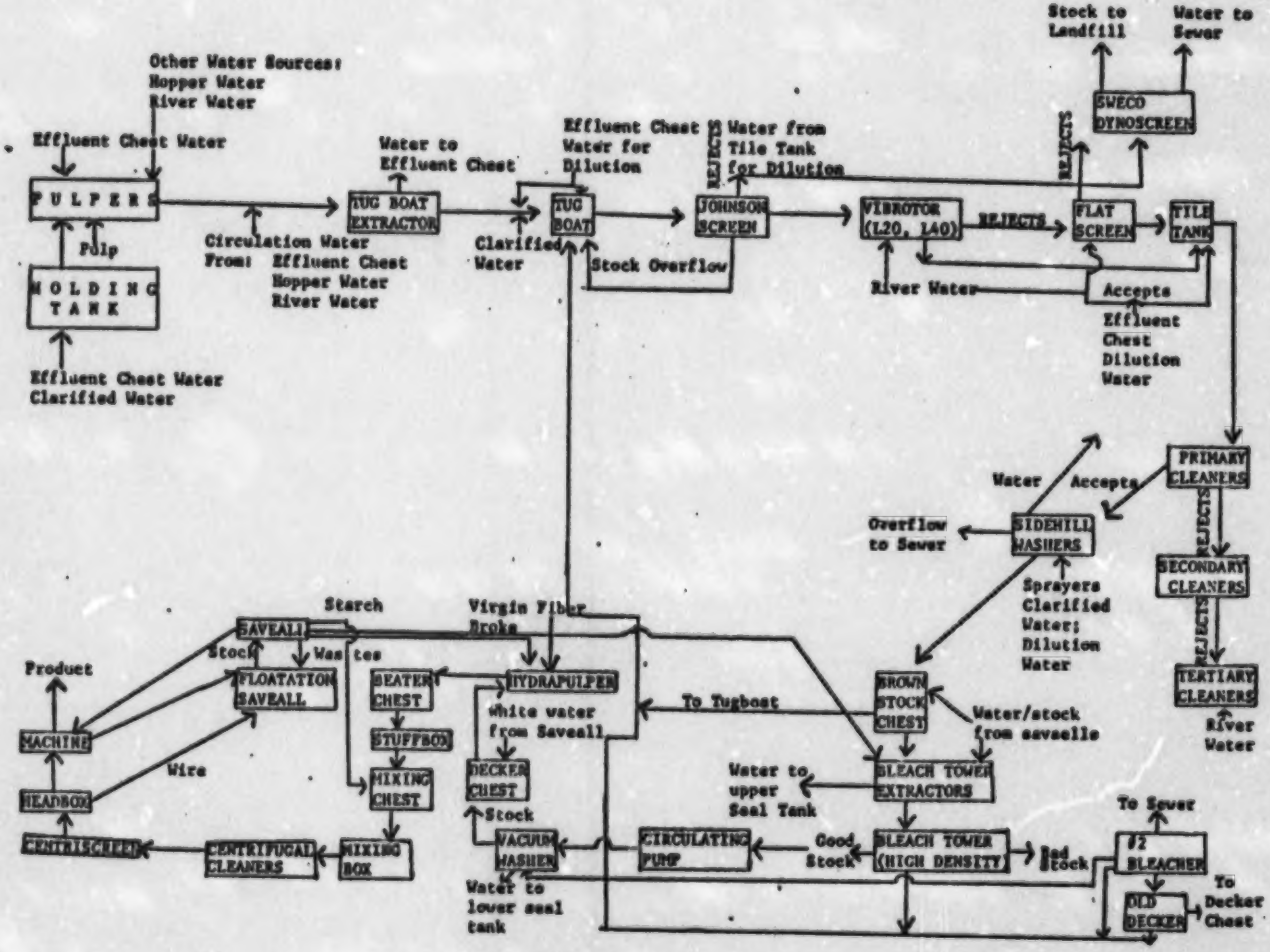

FIGURE 6

SCHEMATIC FLOW DIAGRAM OF A DEINK PULP AND FINE PAPERS PLANT

TABLE 4

FECAL COLIFORM AND KLEBSIELLA RESPONSES OF A WISCONSIN DEINK PULP AND FINE PAPERS PLANT

Water Source	FC/100 ml	FC identified as Klebsiella	% FC identified as Klebsiella	Total Klebsiella /100 ml
Influent water to plant	490	<10		<10
Holding tank	<10	<10		<10
Effluent chest water	145,000	140,000	97	
Water → tile tank	42,000	39,000	93	50,000
Sidehill washers	$2x10^5$ EST	$1x10^5$ EST	50	
Stock → decker chest	19,000	14,000	74	
Decker chest	4,000	3,500	88	
Hydrapulper	<10	<10		<10
Mixing chest	<10	<10		<10
Stock from centri-screen → headbox	<10	<10		<10
Flotation saveall	<10	<10		<10
Stock → 2nd saveall	<10	<10		<10
Primary treatment*	$3x10^5$ EST	$2x10^5$ EST	67	
Secondary treatment*	$2x10^6$ EST	$1x10^6$ EST	50	
Effluent → receiving water	$2x10^6$ EST	$1x10^6$ EST	50	

*Pollution controls: primary clarifier plus 2-stage activated sludge system with holding lagoon for sludges.

reduction. Because the treatment phase of the plant's operational system has no apparent effect in reducing the fecal coliform and Klebsiella bacteria levels in water that is eventually discharged into a receiving body of water, it is clear that effluent water to be reused and stored as effluent chest water would serve as an apparent originating source for a continuous inoculum of coliform bacteria within the mill system.

Figure 7 is a schematic flow diagram depicting a typical sulfite pulp and fine papers plant sampled for FC and Klebsiella response. As before, the continuity of Klebsiella responding as FC positive is clearly apparent. The results of bacterial response from this sulfite mill are shown in Table 5.

Water flowing into the plant had minimum detectable coliform bacteria; however, raw wood with probable soil contamination was the apparent originating source of the FC and Klebsiella bacteria within the plant (Klebsiella accounted for 50% of the FC bacteria isolated). Elevation of FC and Klebsiella bacteria was noted in both the slush and brok pulp. Again, it was found that treated waste water had minimum effect in lowering the bacteria levels and that in fact elevation of bacteria occurred before final discharge of waste water into the receiving body of water. Klebsiella alone accounted for 90% of the isolated FC bacteria. Reuse of any of the treated water could thus serve as a continuous inoculum of Klebsiella and FC bacteria within the plant.

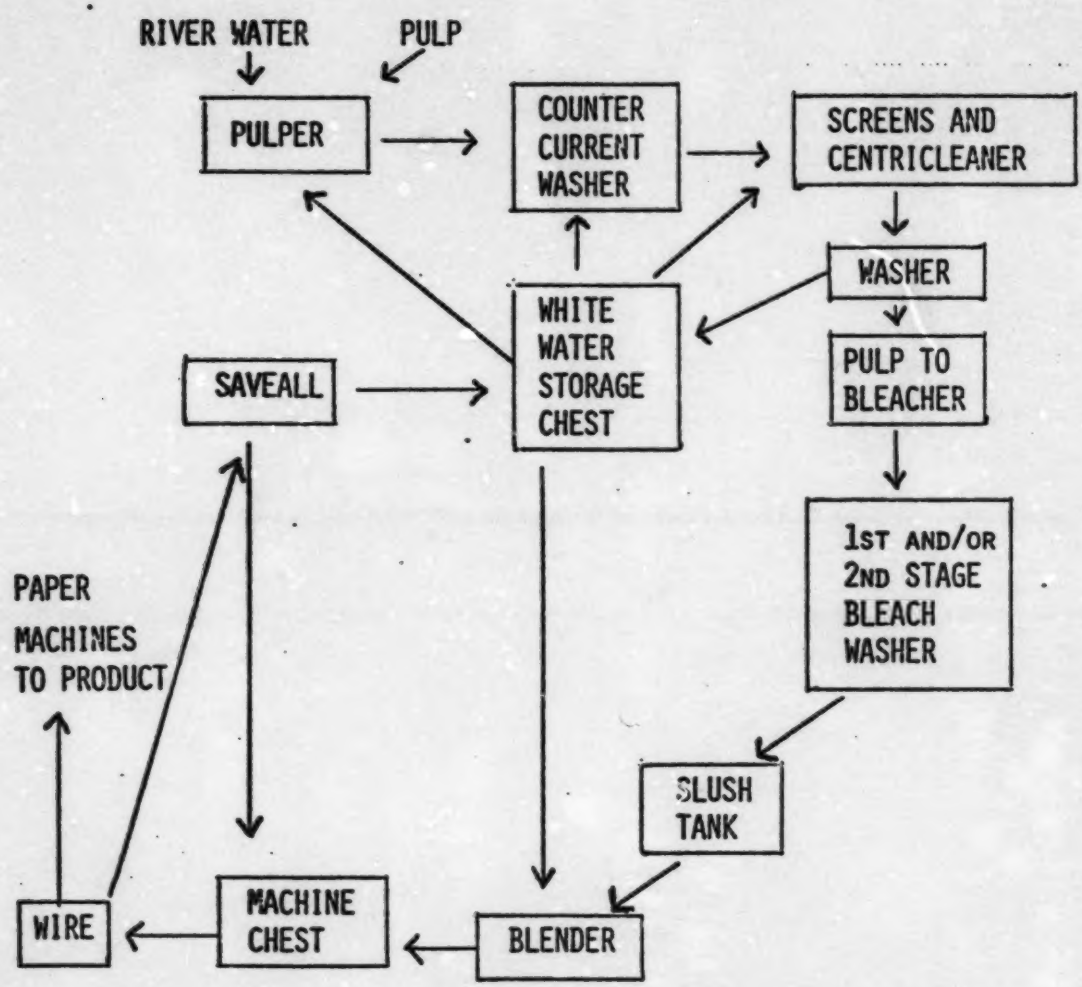

FIGURE 7

SCHEMATIC FLOW DIAGRAM OF A SULFITE PULP AND FINE PAPERS PLANT

TABLE 5

FECAL COLIFORM AND KLEBSIELLA RESPONSES OF SAMPLED WISCONSIN SULFITE PULP AND FINE PAPERS PLANT

Water Source	FC /100 ml	FC identified as Klebsiella	% FC identified as Klebsiella	Klebsiella /100 ml
Influent water → plant	<10	<10		<10
Wood chips* extracted w/sterile phosphate buffer	200			100
Slush pulp	200	100	50	5,000
Brok pulp	700	400	57	7,000
13% spent sulfite liquor	<1,000	<1,000		<1,000
Counter current washer	<100	<100		<100
Bleach washer	<10	<10		<10
Sludge from raw water clarifier	<100	<100		600
White water from #1 & #2 paper machine	550			400
White water from #3 paper machine	<100	<100		<100
Trim off #2 machine	<100	<100		<100
Lift station** (prior to primary treatment)	270,000	100,000	37	$2x10^6$EST
To primary treatment	1,700	1,000	59	40,000
Primary treatment	48,000	40,000	83	670,000EST
After nutrients	47,000	37,000	79	900,000EST
Effluent	50,000	45,000	90	200,000EST

*Hemlock, balsam and birch.

**Pollution controls: primary clarifier, trickling filter, secondary clarifier and mechanized sludge dewatering.

Although sampling from within mills was limited to only three plants, I was able to obtain further information confirming selective fecal coliform growth in recycled or reused water within plants from a 1975 special investigation of a Wisconsin roofing felt plant company whose pollution control mechanism consisted primarily of a clarifier--i.e., ~100% recycle. Table 6 summarizes the FC response from selected areas within the roofing felt plant company. Though FC bacteria in the inflowing river water to the plant are present in low numbers, the wood white water chest serves as an apparent source for FC proliferation. Clarified effluent water that is stored in the white water chest and subsequently reused serves as a continual source of the FC bacteria.

The biochemical test responses of the influent to treatment and effluent water sources of the isolated Klebsiella are summarized in Table 7. The isolates of Klebsiella from the pulp and paper mill industrial effluent that were treated and discharged have very similar biochemical properties to those published by Edwards and Ewing (24).

Tables 8, 9, and 10 summarize the Klebocin biotype patterns and codes for the groundwood pulp mill, deink pulp, and sulfite pulp mills, respectively. For every sample site within each plant, 20 isolated and presumptively identified Klebsiella colonies were picked and streaked onto nutrient agar plates, incubated at 35°C for 24 hr, and shipped to the U.S. EPA Laboratory in West Kingston, Rhode Island for biotyping analysis. Distinct biotype patterns were observed in each plant and from within each plant certain biotypes appeared to

TABLE 6

SUMMARY OF THE FECAL COLIFORM RESPONSE FROM SELECTED AREAS WITHIN THE ROOFING FELT PLANT

Date	Water Source	FC/100 ml
1-18-75	River water influent	150
1-11-75	Influent water before filter treatment	430
1-24-75	Influent water before filter treatment	430
1-11-75	Wood white H_2O chest	<24,000
1-18-75	Wood white H_2O chest	4,600
1-24-75	Wood white H_2O chest	<240,000
1-11-75	Filter effluent	930
1-13-75	Filter effluent	11,000
1-18-75	Filter effluent	210
1-24-75	Filter effluent	430
1-11-75	Clarifier effluent sludge	<24,000
1-18-75	Clarifier effluent sludge	<24,000
1-24-75	Clarifier effluent sludge	<240,000
1-13-75	Filter backwash	<24,000
1-24-75	Filter backwash	11,000
1-11-75	Clarifier effluent water	<24,000
1-18-75	Clarifier effluent water	<24,000
1-24-75	Clarifier effluent water	<240,000

TABLE 7

BIOCHEMICAL REACTIONS OF KLEBSIELLA ASSOCIATED WITH INFLUENT TO TREATMENT AND EFFLUENT WATER SOURCES OF WISCONSIN PULP AND PAPER MILLS AS COMPARED TO DATA OF EDWARDS AND EWING (24)

Test Substrate	Influent to Treatment and Effluent Water Sources Associated with Klebsiella*	Clinical Edwards and Ewing
	% +	% +
Indole	3/30 = 10	6
Methyl red	5/30 = 17	13.3
Vogues proskauer	28/30 = 93	91.1
Simmons' citrate	30/30 = 100	97.7
Urease	29/30 = 97	94.5
Lysine decarboxylase	30/30 = 100	97.2
Ornithine decarboxylase	0/30 = 0	0
Lactose: acid	30/30 = 100	98.2
gas	30/30 = 100	98.0
Motility	0/30 = 0	0

*Includes isolates obtained from all sampled plants.

TABLE 8

KLEBOCIN BIOTYPES FOR THE SAMPLED GROUNDWOOD PULP MILL

Source	Isolated Biotypes
Influent water to plant	32, 40, 812, 908, 1576, 1580, 1836, 3116, 3304, 3372, 3620, 3628, 3692, 3912, 3916, 4076, 4078 Frequency of isolation: 11% for 1576 11% for 3116 5% for all else
Washed wood*	172, 4076, 7918, 7980, 8172, 8174 Frequency: 50% for 8172 25% for 8174 10% for 4076 5% for all else
Grinder white water	4078, 8174 Frequency: 95% for 8174 5% for all else
White water chest	1774, 3072, 3884, 4008, 4076, 4078, 7982, 8172, 8174 Frequency: 35% for 8174 20% for 4076 10% for 4078 5% for all else
Growth on sides of white water chest	108, 1548, 1900, 3820, 4076, 4078, 8172, 8174 Frequency: 39% for 4076 28% for 8172 6% for all else
Thin stock	96, 236, 1024, 3564, 4072, 4076, 8174, 8175 Frequency: 36% for 8174 21% for 8175 7% for all else
Hydrapulper	46, 238, 1516, 1796, 1996, 2028, 2030 Frequency: 53% for 2030 18% for 238 6% for all else

TABLE 8 (continued)

Source	Isolated Biotypes
Wet machine	1518, 1806, 2020, 2028, 2030, 4078 Frequency: 58% for 2030 16% for 1518 11% for 2028 5% for all else
Clarified white water	12, 556, 1792, 4076 Frequency: 25% for each

*Aspen.

TABLE 9

KLEBOCIN BIOTYPING RESULTS OF SAMPLED WISCONSIN DEINK PULP AND FINE PAPERS PLANT

Water Source	Isolated Klebocin Biotypes
Effluent chest water	4070, 6126, 7936, 8110, 8132, 8164, 8174, 8175 Frequency: 58% for 8174 8% for 8110 8% for 8175 4% for all else
Water into tile tank	6126, 7942, 7963, 8100, 8108, 8110, 8174 Frequency: 58% for 8174 13% for 6126 4% for all else
Sidehill washers	7936, 7940, 8166, 8172, 8174, 8190 Frequency: 42% for 8174 17% for 8172 17% for 8190 8% for all else
Stock into decker chest	8164, 8172, 8174, 8175, 8190 Frequency: 40% for 8174 30% for 8172 10% for all else
Decker chest	238, 6124, 6126, 8172, 8174 Frequency: 45% for 8172 40% for 8174 5% for all else
Primary treatment	44, 1068, 1196, 1964, 3756, 5676, 8108, 8172, 8174 Frequency: 11% for each
Secondary treatment	1896, 3884, 3948, 4012, 4076, 8172, 8174 Frequency: 30% for 4076 20% for 3948 10% for all else
Effluent	1192, 1548, 3756, 3820, 5292, 7636, 7916, 7936, 7980 Frequency: 20% for 7980 10% for all else

TABLE 10

KLEBOCIN BIOTYPING RESULTS FROM SAMPLED WISCONSIN SULFITE PULP AND FINE PAPERS MILL

Water Source	Isolated Klebocin Biotypes
Washed raw wood*	6143 Frequency: 100%
Slush pulp	44, 246, 256, 1024, 1280, 1536, 1792, 1856, 1936, 1996, 2024, 2028, 2030 Frequency: 12% for 2028 12% for 2030 12% for 1280 6% for all else
White water from #1 & #2 paper machines	238, 2030, 6126 Frequency: 60% for 2030 30% for 6126 10% for 238
Prior to primary treatment	1564, 1796, 1804, 1816, 1820, 2030, 6142 Frequency: 35% for 6142 20% for 1820 10% for 2030 10% for 1804 10% for 1796 5% for all else
To primary treatment	238, 2030, 6126 Frequency: 60% for 2030 30% for 6126 10% for 238
Primary effluent	256, 1792, 1796 Frequency: 55% for 1792 36% for 1796 9% for 256
After nutrients	16, 1040, 1536, 1552, 1820, 1848, 1852 Frequency: 37% for 1852 32% for 1552 11% for 1848 5% for all else
Effluent	552, 768, 1536, 1544, 1580, 1792, 1832 Frequency: 30% for 1792 20% for 1832 10% for all else

*Hemlock, balsam, and birch.

be selectively present from start to finish of the mill processing systems. The Klebocin sensitivity typing of Klebsiella incorporated a battery of 13 tests with resulting 2^{13} or 8192 patterns having assigned numbers from 0 to $2^{13}-1$ or 0-8191 (see Table 11 for comparison of biotypes from each plant).

From Table 8 it is apparent that the white water chest serves as the reservoir for maintaining the biotypes initially inoculated from the influent and washed wood sources. From the white water chest the biotypes are distributed further into subsequent processing stages and maintained throughout the complete mill processing system. Following type 4076 from the influent through to the final clarified white water source exemplifies this. The frequency of isolating type 4076 is 5% of the total isolated biotypes from influent water, 10% from washed raw wood, 20% from the white water chest, 35% from the growth on sides of the white water chest, and 5% throughout the remaining processes. Note that the highest frequency of type 4076 isolation occurs at the white water chest.

From Table 9 it is apparent that the effluent chest water serves as a reservoir for maintaining a variety of Klebocin biotypes which are subsequently distributed into later processing stages where their proliferation is thereby enhanced. Biotype 8174 from the effluent chest water serves as a good example for illustrating the continued presence of a particular Klebocin biotype throughout the mill because of the continued reuse or recycling of this water source. The frequency of isolating type 8174 is 58% from the effluent chest

TABLE 11

TOTAL KLEBOCIN BIOTYPES ISOLATED
FROM THE THREE SAMPLED PLANTS

Sulfite Pulp and Fine Papers Plant 32 - water 17 - sewage	Groundwood Pulp Mill 59 - water 0 - sewage 1 - nose	Deink Pulp and Fine Papers Plant 36 - water 0 - sewage
16	12	
	32	
	40	
44		44
	46	
	96	
	108	
	172	
	236	
238		238
246	364	
256	556	
552	812	
768	908	
1024	1024	
	1068	1068
1040	1516	1192
1280	1518	1196
1536		
1544	1544	
	1548	1548
1564	1576	
1580	1580	
*S1668	1774	
S1732		
S1774		
S1788		
1791		
1792	1792	
S1792		
1796	1796	
S1796		
1804	1806	1896
S1804	1836	
1816	1900	
1820		
1832		
1848		
1852		

TABLE 11 (continued)

Sulfite Pulp and Fine Papers Plant 32 - water 17 - sewage	Groundwood Pulp Mill 59 - water 0 - sewage 1 - nose	Deink Pulp and Fine Papers Plant 36 - water 0 - sewage
1856		
S1920		
S1924		
S1928		
1936		
S1940		
S1948		
S1964		1964
S1980		
1996	1996	
2024	2020	
2028	2028	
S2028		
2030	2030	
S2044	3072	3756
S2046	3116	
	3304	
	3372	
	3564	
	3620	
	3628	
	3648	
	3656	
	3692	
	3820	3820
	3884	3884
	3912	
	3916	
	3948	3948
	4008	4012
	4072	4070
	4076	4076
	4078	5292
	5868	5676
		6124
6126		6126
6142	7788	7636
6143	7918	7916
		7936
		7940
		7942
		7963

TABLE 11 (continued)

Sulfite Pulp and Fine Papers Plant 32 - water 17 - sewage	Groundwood Pulp Mill 59 - water 0 - sewage 1 - nose	Deink Pulp and Fine Papers Plant 36 - water 0 - sewage
	7980	7980
	7982	8100
		8108
		8110
		8132
		8164
		8166
	8172	8172
	8174	8174
	8175	8175
	**N8175	8190

*S refers to sewage samples.

**N refers to human nasal source.

water, 58% from the tile tank, 42% from the side hill washers, 40% from stock into decker chest, 40% from the decker chest, 11% from primary treatment, and 10% from secondary treatment. Note that the highest frequency of biotype 8174 isolation occurred at the effluent chest water source.

Though the total Klebsiella counts detected in the white water system of the sulfite pulp and fine papers plant were relatively low (Table 5), it is apparent that the white water system still functions as a reservoir for certain Klebsiella types that get distributed into later processing portions within the plant. Biotype 2030 exemplifies the continuity of a specific biotype present in waters reused or recycled into later processing portions of the plant. The frequency of isolating type 2030 was 60% in the white water from #1 and #2 paper machines, 20% in the slush pulp, 20% in water prior to primary treatment, and 60% in water to primary treatment. Note identical frequency and biotype characterization between white water from #1 and #2 paper machines and water to primary treatment. In all three cases, though the plant processing and isolated biotypes varied greatly, there still remained continuity with respect to isolating the highest frequency of specific Klebsiella biotypes in portions of the plants that reused or recycled water.

I was able to sample the upper respiratory tract of workers in the groundwood pulp mill for Klebsiella carriage. Workers were asked to fill out a general information questionnaire (see Appendix A) to categorize themselves according to age, length of employment,

residence, previous employment, smoking history, and use of alcoholic beverages as a means of matching the paper workers to control workers. Workers were also asked to fill out and sign a consent form indicating their agreement for participation with the study (see Appendix B).

A total of 24 plant employees were sampled for Klebsiella carriage via nose and throat swab cultures and Klebsiella was recovered from the nasal passage of one worker from the groundwood pulp mill. Of the 24 employees, 9 were clerical and managerial positions while 15 persons were involved with direct mill processing jobs. The work categories, employment histories, and Klebsiella carriage for each age and sex are shown in Table 12.

To obtain a control rate of Klebsiella carriage for comparison, I was able to sample a group of workers from a city park and recreation department located in a geographically comparable area to the sampled paper workers. From the 27 control workers studied, I was not able to isolate any Klebsiella from the upper respiratory tract. The employment, age, sex, and Klebsiella carriage of each worker is shown in Table 13.

Klebsiella enumeration from the nasal passage of worker #12 (Table 12) exceeded 400 organisms per nasal swab.* The worker was employed in a maintenance lead position where he had contact with all of the pulping process stages. Twenty isolated colonies were picked

*Approximately 0.1 ml extracted per swab.

TABLE 12

EMPLOYMENT CATEGORIES, EMPLOYMENT HISTORIES, AGE, SEX, AND *KLEBSIELLA* CARRIAGE OF WORKERS SAMPLED FROM WISCONSIN GROUNDWOOD PULP MILL*

Work Area	Worker Number	Age	Sex	Length of Employment	*Klebsiella* Carriage Nasal	Throat
	1	34	M	10 years	no	no
	2	31	F	3.5 years	no	no
Office-related	3	28	M	3 years	no	no
	4	48	M	3 years	no	no
	5	25	F	2 years	no	no
	6	27	M	.5 years	no	no
	7	29	M	4 months	no	no
Maintenance and Janitorial	8	36	M	4 years	no	no
	9	27	M	2 years	no	no
	10	78	M	40 years	no	no
	11	47	M	30 years	no	no
	12	51	M	30 years	yes	no
	13	47	M	25 years	no	no
	14	60	M	10 years	no	no
	15	41	M	10 years	no	no
	16	34	M	5 years	no	no
Mill-related	17	24	M	4 years	no	no
	18	24	M	3 years	no	no
	19	23	M	3 years	no	no
	20	34	M	2.5 years	no	no
	21	22	M	2 years	no	no
	22	29	M	8 months	no	no
	23	18		3.5 months	no	no
	24	25		2.5 months	no	no

*Mean age=35 with range from 18 to 75 years; mean length of employment=8.1 years with range from 40 years to 2.5 months.

TABLE 13

EMPLOYMENT, AGE, SEX, AND KLEBSIELLA CARRIAGE OF THE SAMPLED WISCONSIN PARKS AND RECREATION DEPARTMENT CONTROL WORKERS*

Worker Number	Age	Sex	Length of Employment	Klebsiella Carriage	
				Nose	Throat
1	55	M	33 years	no	no
2	54	M	21 years	no	no
3	58	M	20 years	no	no
4	53	M	18 years	no	no
5	48	M	17 years	no	no
6	43	M	17 years	no	no
7	44	M	15 years	no	no
8	56	M	16 years	no	no
9	59	M	13 years	no	no
10	55	M	13 years	no	no
11	32	M	12 years	no	no
12	34	M	12 years	no	no
13	36	M	11 years	no	no
14	38	M	10 years	no	no
15	29	M	9 years	no	no
16	31	M	9 years	no	no
17	36	M	9 years	no	no
18	35	M	8.5 years	no	no
19	37	M	5 years	no	no
20	23	M	4 years	no	no
21	30	M	4 years	no	no
22	28	M	3 years	no	no
23	29	M	2.5 years	no	no
24	52	M	1.5 years	no	no
25	26	M	1 years	no	no
26	23	M	3 weeks	no	no
27	33	M	1 week	no	no

*Mean age=39.8 years with range from 23 to 58 years; mean length of employment=10.5 years with range from 1 week to 33 years.

from the EMB plates and presumptively identified as Klebsiella via the biochemical test responses. Table 14 summarizes the biochemical response pattern for the 20 isolates of Klebsiella carried in the nasal passage of the groundwood pulp worker. The biochemical patterns are in very close correlation to those recommended for Klebsiella identification according to Edwards and Ewing (24).

Klebocin biotyping was performed on 10 of the isolated Klebsiella colonies from the human nasal specimen and the results are shown in Table 15. Klebocin biotype 8175 was the only biotype isolated. Klebocin biotype 8175 was also found in the groundwood pulp mill's thin stock water supply (Table 8) with an isolation frequency of 21%. The human nasal specimen from worker #12 (Table 12) also correlates to the same biotype (8175) that was found in the effluent chest water (frequency of 8%) of the sampled deink pulp and fine papers plant (Table 9).

The finding that the worker carried Klebsiella in the nasal passage is of interest in itself, since nasal carriage in nonhospitalized populations has not been closely studied although one report indicated that prevalence rates for such colonization are low (26). Isolation of Klebsiella in the oropharynx has not been completely studied since Klebsiella is not considered to be a member of the normal resident mouth flora.

The findings were limited to a small group of workers where only 1 out of 15 employed in direct mill processing was positive for Klebsiella isolation. Examination of the control group consisting

TABLE 14

BIOCHEMICAL RESPONSES FOR THE 20 ISOLATES OF HUMAN NASAL SOURCE KLEBSIELLA

Test Substrate	Number +	Percent +
Indole	2/20	10
Methyl red	3/20	15
Vogues proskauer	18/20	90
Simmons' citrate	20/20	100
Urease	19/20	95
Lysine decarboxylase	20/20	100
Ornithine decarboxylase	0/20	0
Lactose: acid	20/20	100
gas	20/20	100
Motility	0/20	0

TABLE 15

KLEBOCIN BIOTYPING RESULTS OF 10 ISOLATED HUMAN NASAL SPECIMENS OF KLEBSIELLA

Source	Isolated Biotype
Human nasal specimen	8175 Frequency: 10/10 or 100%

of 27 workers, whose work also involves possible environmental-source exposure, did not confirm any Klebsiella isolations.

Because of difficulties in getting more plants to cooperate with sampling workers within plants, several plant sanitary sewers were evaluated for fecal coliform and Klebsiella responses. Table 16 summarizes the results from the plant sanitary sewer samples of the groundwood pulp mill and the sulfite pulp and fine papers plant.

As apparent from Table 16, several of the Klebsiella biotypes isolated from the plants' sanitary sewers matched with several of the types isolated from within the plant's processing waters (see Table 10). Though gastrointestinal carriage of Klebsiella is suspect, the exact source cannot be determined without actual human specimen evaluation.

TABLE 16

FECAL COLIFORM AND KLEBSIELLA RESPONSES FROM TWO PLANT SANITARY SEWER SYSTEMS

Source	Fecal Coliform per 100 ml	Fecal Coliforms Identified as Klebsiella per 100 ml	% FC Identified as Klebsiella	Klebsiella Biotypes Isolated
Groundwood pulp mill	80,000	24,000	30	
Sulfite pulp & fine papers	260,000	110,000	55	1668,1732 1774, 1788 1792,1796 1804, 1920 1924, 1928 1940, 1948 1964, 1980 2028, 2044 2046

V. DISCUSSION

In general, the results showed that the level of fecal coliform bacteria present in influent waters supplying paper mills, as measured by the standard fecal coliform indicator test, was significantly lower than the fecal coliform counts being discharged from plant effluents. In two out of the three mills sampled, the influent water source had minimum detectable levels of fecal coliform bacteria (i.e., <10 organisms per 100 ml) whereas effluent water discharged had high levels of fecal coliform bacteria ranging from 270,000 to an estimated 2×10^6 organisms per 100 ml. Since Klebsiella was the predominant coliform bacteria identified as giving a positive fecal coliform response, it may be postulated that Klebsiella proliferating early in the pulp processing stages may be responsible for the elevated fecal coliform levels discharged from plants in the final effluent. The study observations give supporting evidence for Klebsiella maintaining a possible wood or bark reservoir since influent water supplying the three sampled mills had no detectable Klebsiella levels. However, once raw wood combined with distilled phosphate buffer was sampled from each plant, Klebsiella was consistently isolated.

Thus, once Klebsiella proliferated from the early pulping stages, the reuse of water within the plants aided in maintaining elevated bacterial levels through each plant's treatment stages.

Wastewater treatment* could not significantly reduce the Klebsiella nor total fecal coliform levels.

The goal of a fecal coliform indicator implicitly refers to a restricted group of microorganisms specifically and exclusively associated with fecal wastes. However, the study findings indicate that such is not the case because of the significant proportion of Klebsiella bacteria isolated from pulp and paper mill processing waters having no apparent fecal source. The fecal coliform test selects for bacteria primarily of the genus Escherichia but because of the test's lack of specificity, other bacteria including Klebsiella, Citrobacter, and Enterobacter give positive reactions as well. Because of Klebsiella's tremendous "regrowth" potential in waters contaminated with pulp and paper mill waters, there is an increasing need for reevaluating the validity of the fecal coliform test as an indicator in these types of industrial wastewaters.

Since Klebsiella is not exclusively or specifically associated with fecal waste, the study findings recommend that highly specific indicators of fecal waste contamination, such as E. coli, be used as indicators of fecal contamination, thereby eliminating the interfering response of Klebsiella bacteria from a nonfecal source.

Further study is needed in the evaluation of the health effects in workers exposed to high levels of Klebsiella in the workplace. The study findings indicated that paper industry processes

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*Two plants maintained primary and secondary treatment facilities while one operated a primary clarifier.

selectively allowed Klebsiella to grow and in levels exceeding the only water contact standard promulgated--i.e., fecal coliform levels cannot exceed 200 organisms per 100 ml. Klebsiella and fecal coliform bacteria were present in levels as high as an estimated 2×10^6 organisms per 100 ml where exposure to any worker involved in the mill processing could occur. Although no significant relationship was found for Klebsiella colonization in paper workers, there is a strong suggestion that upper respiratory tract colonization can occur and larger sample size evaluation with subsequent infection follow-up may answer the question of potential health hazards to pulp and paper mill workers occupationally exposed to Klebsiella.

REFERENCES

1. Geldreich, E. E. 1966. Sanitary significance of fecal coliforms in the environment. U.S. Dept. of the Interior, Federal Water Pollution Control Administration. Publication WP-20-3, U.S. Government Printing Office, Washington, DC.

2. Blosser, R. O. 1971. Experience with indicator organism tests in determining the bacteriological quality of pulp and paper mill effluents and their receiving waters. National Council of the Paper Industry for Air and Stream Improvement Tech. Bull. No. 244, p. 56.

3. Dutka, B. J., J. B. Bell, P. Collins, and J. Popplow. 1969. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Div. Publ. Health Eng. Manuscript Rep. KR-69-1, p. 42.

4. Reid, J. E., A. D. Tennant, and L. J. Rockwell. 1961. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Lb. Hyg. Manuscript Rep. 51-1, p. 51.

5. Reid, J. E., A. D. Rennant, and L. J. Rockwell. 1963. A bacteriological study of the Saint Croix River, International Joint Commission. Department of National Health and Welfare (Canada). Lab. Hyg. Manuscript Rep. 63-1, p. 34.

6. Tennant, A. D., J. E. Reid, and L. J. Rockwell. 1960. A bacteriological study of the Rainy River, conducted for the Advisory Board on Water Pollution, Rainy River and Lake of the Woods, International Joint Commission. Department of National Health and Welfare (Canada). Lab. Hyg. Manuscript Rep. 60-1, p. 52.

7. Calkin, J. B. 1957. Modern pulp and papermaking. 3rd edition, Reinhold Publishing Corp., New York.

8. Huntley, B. E., A. C. Jones, and V. J. Cabelli. 1976. Klebsiella densities in waters receiving wood pulp effluents. Journal Water Pollution Control Federation, 48-7, p. 1766.

9. Watkins, S. H. 1973. Coliform bacteria growth and control in aerated stabilization basins. Environmental Protection Agency, 660/2-73-028 (December).

10. Thoburn, R., F. R. Fekety, L. E. Cluff Jr., and V. B. Melvin. 1968. Infections acquired by hospitalized patients. Arch. Intern. Med. (Chicago), 121:1.

11. Finland, M., W. F. Jones Jr., and M. W. Barnes. 1959. Occurrence of serious bacterial infections since introduction of antibacterial agents. Journal of the American Medical Association 170:2188.

12. Dunca, D. W. and W. E. Razzell. 1972. Klebsiella biotypes among coliforms isolated from forest environments and farm produce. Applied Micro. 24:933.

13. Borner, R. H. and B. J. Carrul. 1972. Proceedings seminar on the significance of fecal coliforms in industrial wastes. TR-3-U.S. Environmental Protection Agency.

14. Knittel, M. D., R. J. Seidler, C. Eby, and L. M. Cabe. 1977. Colonization of the botanical environment by Klebsiella isolates of pathogenic origin. Applied Micro. 34:557.

15. National Council of the Paper Industry for Air and Stream Improvement Tech. Bull. No. 279, March 1975. Further studies of the -anitary significance of K. pneumoniae occurrence in mill effluents and surface waters.

16. Mallmann, W. L. 1970. Conference in the matter of pollution of the navigable waters of Mobile Bay and its tributaries--Alabama. Federal Water Pollution Control Administration, U.S. Dept. of the Interior, p. 263.

17. Huntley, B. E., A. C. Jones, and V. J. Cabelli. 1976. Klebsiella densities in waters receiving wood pulp effluents. Journal of the Water Pollution Control Federation 48:1766.

18. Seidler, R. J., T. E. Morrow, and S. T. Bagley. 1977. Klebsiella in drinking water emanating from redwood storage tanks. Applied Micro. 33:893.

19. Dufour, A. D. and V. J. Cabelli. 1976. Characteristics of Klebsiella from textile finishing plant effluents. Journal of the Water Pollution Control Federation 48:872.

20. Bagley, S. T. and R. J. Seidler. 1977. Significance of fecal coliform-positive Klebsiella. Appl. Environ. Microbio. 33:1141.

21. American Public Health Association. ETM:Standard Methods for the Examination of Water and Wastewater. 1975. 14th ed., Amer. Pub. Health Assoc., Inc., New York.

22. Dufour, A. P. and L. B. Lupo. 1977. A membrane filter method for enumerating Klebsiella species. U.S. EPA Marine Field Station, RI Annual Meeting, American Society for Microbiology.

23. Buffenmyer, C. L., R. R. Rycheck, and R. B. Yee. 1976. Bacteriocin (Klebocin) sensitivity typing of Klebsiella. Journal of Clinical Microbiology 4(3):239.

24. Edwards, P. R. and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.

25. Vlassoff, L. T. 1977. Klebsiella bacterial indicators/Health Hazards Associated with Water. Hoadley/Dutka, eds., p. 275.

26. Montgomerie, J. Z. 1979. Epidemiology of Klebsiella and hospital-associated infections. Review of Infect. Dis. 1(5): 736.

APPENDIX A

SAMPLE QUESTIONNAIRE

Identification number_____

Age_____ Marital status_____

Sex_____ Children_____

Race_____

Residence_____

Length of residence_____

Job title_____

Job description_____

Length of employment_____

Previous job_____ Date_____

Location_____

Job description_____

_____

Have you ever smoked?_____

Do you smoke now?_____

	Age started	Age stopped	How many
Cigarettes	_____	_____	_____/packs
Pipe	_____	_____	_____/day
Cigars	_____	_____	_____/day

Do you presently use alcoholic beverages?_____

If yes, how much do you drink on an average per week?_____

APPENDIX B

SAMPLE CONSENT FORM

You are invited to participate in a research project which will aid in the determination of the importance of a specific bacteria referred to as "Klebsiella" in pulp and paper mill workers. Participation in this study is strictly voluntary and you may terminate your consent and discontinue participation in the project at any time without prejudice to youerself. The study is not likely to benefit you directly, though it may increase our knowledge of one kind of colonization in paper mill workers.

The workers are asked to provide general information for a brief questionnaire to be administered and to allow for nose and throat swabs to be obtained. The nose swab is unpleasant for a few seconds, but discomfort usually disappears soon after.

Participating workers are assured of complete confidentiality of any recorded information. The results from this research project will be disclosed to any interested participants after the study is completed. Before you sign this form, please ask any question on any aspect of this study that is unclear to you.

AUTHORIZATION: I, _____ have read
(name of subject)
the above and decided to participate in the research project described above. My signature also indicates that I have received a copy of this consent form.

_____ _____
(Signature) (Date)

_____ _____
(Signature of principal investigator) (Telephone)